DATA EVALUATION RECORD
ACUTE EC_{50} TEST WITH AN ESTUARINE/MARINE MOLLUSK
SHELL DEPOSITION STUDY

1. **CHEMICAL:** Ethylenethiourea — PC Code No.: 600016

2. **TEST MATERIAL:** Ethylenethiourea — Purity: 100%

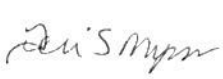
3. **CITATION**

Authors:	York, D. O.
Title:	Ethylenethiourea- Acute Toxicity to Eastern Oyster (*Crassostrea virginica*) Under Flow-Through Conditions, Following OPPTS Guideline (Draft) 850.1025
Study Completion Date:	June 12, 2008
Laboratory:	Springborn Smithers Laboratories, Wareham, Massachusetts
Sponsor:	EBDC/ETU Task Force, c/o McDermott, Will and Emery LLP, Washington, DC
Laboratory Report ID:	13921.6102
MRID No.:	47474301
DP Barcode:	D360293

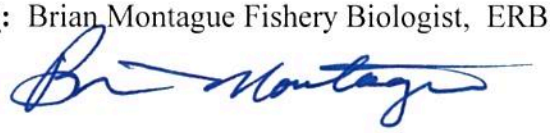
4. **REVIEWED BY:** John Marton, Staff Scientist, Cambridge Environmental, Inc.

Signature:  **Date:** 09/01/09

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental, Inc.

Signature:  **Date:** 09/24/09

5. **APPROVED BY:** Brian Montague Fishery Biologist, ERB5/EFED/OPP/OSCPP/USEPA

Signature:  **Date:** 3/4/15

Secondary Reviewer:  **Date:** 04/16/15

6. **DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to shell deposition in oysters. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if

appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study

7. STUDY PARAMETERS

Age or Size of Test Organism:	mean valve height: 44±2.8 mm, N=30
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured

8. CONCLUSIONS:

Results Synopsis

EC_{50}: >110 mg ai/L 95% C.I.: N/A
NOAEC: 42 mg ai/L
Probit Slope: N/A

9. ADEQUACY OF THE STUDY

A. Classification: Acceptable
B. Rationale: Observed results do support the conclusions of the study director
C. Repairability:

10. BACKGROUND

11. GUIDELINE DEVIATIONS: This study was conducted following guidelines outlined in U.S. Environmental Protection Agency's Ecological Effects Test Guideline OPPTS (Draft) Guideline 850.1025 and OPPTS 850.1000 Special Consideration for Conducting Aquatic Laboratory Studies.

1. Though only minimal growth was achieved in the control oysters there was a reduction noted in all other treatment groups except for one.
2. Though an EC50 was not obtained, the highest concentration did exceed normally required maximum levels to show the chemical to be practically non-toxic (100 PPM) to eastern oysters. Percent reduction was highest in the two highest concentrations (25 to 30% reductions)

12. SUBMISSION PURPOSE: This study was conducted to provide data on the effects of ethylenethiourea on the shell deposition of Eastern oysters (*Crassostrea virginica*) for the purpose of chemical re-registration.

13. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the Pacific oyster (*Crassostrea gigas*) and the Eastern oyster (*Crassostrea virginica*)	*Crassostrea virginica*
Mean valve height 25 - 50 mm along the long axis	44 mm (SD 2.8 mm), N=30
Supplier	Circle C Oysters, Ridge, Maryland
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days	12 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Amount of peripheral shell growth removed prior to testing	3-5 mm
Feeding during the acclimation Must be fed to avoid stress.	Fed a supplementary algal diet of *Tetraselmus maculate* prepared in

Guideline Criteria	Reported Information
	seawater from a concentrate.
Pretest Mortality <3% mortality 48 hours prior to testing	0% mortality 7 days prior to testing.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Natural unfiltered seawater from an uncontaminated source.	Natural seawater collected from the Cape Cod Canal, Bourne, Massachusetts, filtered to 20‰ with fresh well water.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰	19-20‰
Water Temperature 15E-30E C, consistent in all test vessels	20-22°
pH	7.6-7.9
Dissolved Oxygen ∃ 60% throughout	7.0-7.7 mg/L; 60% saturation value at 21°C with a salinity of 19‰ is 4.8 mg/L; and 60% saturation value at 22°C with a salinity of 19‰ is 4.7 mg/L
Total Organic Carbon	1.2 mg/L
Test Aquaria Should be constructed of glass or stainless steel.	Glass aquaria containing approximately 18 L of test solution.
Type of Dilution System	Flow-through system with an FMI pump

Guideline Criteria	Reported Information
Must provide reproducible supply of toxicant	calibrated to deliver 45 mL/minute of the 1.0 mg/mL diluter stock solution to the mixing chamber. Continuous flow was provided with a 90% solution replacement rate of approximately 8 hours. Additionally, the contents of each aquarium were continuously circulated by pumping the exposure solution with a magnetic drive pump from one end of the aquarium and returning it to the opposite end.
Flow rate Consistent flow rate	6.0 vol/24 hours
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Yes
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark and sudden transitions were avoided
Solvents Not to exceed 0.5 ml/L	Solvent: N/A Maximum conc.: N/A

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L with 30 or more oysters, then no definitive test is required.	A preliminary range-finding study was conducted under flow-through conditions with nominal concentrations of 0 (negative control), 0.012, 0.12, 1.2, 12 and 120 mg ai/L. Thirty oysters (15 per rep) were exposed to each treatment for 96 hours. At test termination, reductions in shell growth of 0, 17 and 73% were observed at the 1.2, 12 and 120 mg ai/L treatment levels, respectively, and the remaining treatment levels showed a positive response compared to the control. No mortalities or adverse effects were observed.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	0 (negative control), 9.3, 16, 26, 43, 72, and 120 mg ai/L
Number of Test Organisms Minimum 20 individual per test level and in each control	40, equally divided among two replicates
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured hourly in at least one chamber 2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low	1. Temperature was measured daily in every test vessel. Temperature was also continuously measured in replicate A of the control. 2. DO and pH were measured daily in every

Guideline Criteria	Reported Information
doses and in the control	test vessel.
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes. Samples were collected at 0 and 96 hours and analyzed using HPLC/UV.

14. <u>REPORTED RESULTS</u>

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. This study was conducted in compliance with all pertinent U.S. EPA Good Laboratory Practice Regulations (40 CFR, Part 160) with the following exceptions: routine water screening analyses for pesticides, PCBs and toxic metals were conducted at GeoLabs, Inc., Braintree, Massachusetts using standard U.S. EPA procedures.
<u>Control Mortality</u> Not more than 10% of control organisms may die or show abnormal behavior.	0 %
<u>Control Shell Deposition</u> Must be at least 2 mm.	2.0 mm
<u>Recovery of Chemical</u>	95-160% of nominal
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Shell Growth

Concentration (mg ai/L)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Reduction
Nominal	Mean Measured				
Control	<LOQ	40	0	2.0	--
9.3	15	40	0	1.7	15
16	22	40	0	1.7	15
26	31	40	0	2.0	0
43	42	40	0	1.7	15
72	68	40	0	1.4	30*
120	110	40	0	1.5	25*

*significantly different from control based on Williams' test.

B. Statistical Results

Method: No inhibitions exceeded 50% relative to the negative control; therefore, the EC_{50} value was visually determined to be greater than the highest mean-measured concentration. The NOAEC value was determined using the Williams test.

96-hr EC_{50}: >110 mg ai/L 95% C.I.: N/A
NOAEC: 42 mg ai/L
Probit Slope: N/A

15. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Statistical Method for EC_{50}	Visual determination
EC_{50} (95% C.I.)	>110 mg ai/L
Probit Slope	N/A

Parameter	Result
Statistical Method for NOAEC	Kruskal-Wallis
NOAEC	42 mg ai/L

16. REVIEWER'S COMMENTS:

The reviewer's results agreed with those of the study author.

High analytical recovery was obtained at the three lowest treatment levels (120-160% of nominal). The study author attributed these high recoveries to the constant flow serial diluter, which tends to yield higher recoveries in the low concentration. However, concentrations appeared stable between 0 and 96 hours. Quality control samples yielded recoveries at 99.2-107% of nominal, indicating that precision and quality control were maintained during the analysis of the test solutions.

The reviewer's analysis of the shell deposition data did not detect significant differences at the two highest treatment levels relative to the control. The reviewer feels that this was due to the lower statistical power of the non-parametric Kruskal-Wallis test. However, the reviewer feels that the 30 and 25% inhibitions at the mean-measured 68 and 110 mg ai/L treatment levels, respectively, were biologically significant and visually determined the NOAEC value to be 42 mg ai/L.

An initial definitive toxicity test was initiated on April 17, 2008 but was terminated before 96 hours due to a technician error. The final definitive test was conducted from May 8 to May 12, 2008.

References:

ASTM. 2002. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Road, West Conshohocken, PA 19428.

Benoit, D.A., V.R. Mattson and D.L. Olson. 1982. A continuous-flow mini-diluter system for toxicity testing. Water Research: 16, 457-464.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish. 1. Bioassay methods for acute toxicity. Water Research: 3, 792-821.

U.S. EPA. 1985. Office of Pesticide Programs. Pesticide Assessment Procedure for Acute Toxicity Test for Estuarine and Marine Organisms (Mollusc 96-Hour Flow-Through Shell Deposition Study) EPA-540/9-85-011. June 1985. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1989. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160): FR: 8/17/89; pp. 34052. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1996a. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Test Guideline, OPPTS 850.1025. Oyster Acute Toxicity Test (Shell Deposition). "Public Draft". EPA 712-C-96-115. April 1996. U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1996b. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Guideline, OPPTS 850.1000. Special Consideration for Conducting Aquatic Laboratory Studies. "Public Draft". EPA 712-C-96-113. April 1996. U.S. Environmental Protection Agency, Washington, DC.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Shell deposition (mm), 96 hrs; mg ai/L
File: 4301sd Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

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INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.938	3.388	5.348	3.388	0.938
OBSERVED	0	5	4	5	0

Calculated Chi-Square goodness of fit test statistic = 3.7497
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Shell deposition (mm), 96 hrs; mg ai/L
File: 4301sd Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.200

W = 0.976

Critical W (P = 0.05) (n = 14) = 0.874
Critical W (P = 0.01) (n = 14) = 0.825

Data PASS normality test at P=0.01 level. Continue analysis.

Shell deposition (mm), 96 hrs; mg ai/L
File: 4301sd Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has
zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

Shell deposition (mm), 96 hrs; mg ai/L
File: 4301sd Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	1.950	1.950	21.500
2	15	1.750	1.750	18.500
3	22	1.700	1.700	16.000
4	31	1.950	1.950	24.000
5	42	1.700	1.700	15.000
6	68	1.400	1.400	4.000
7	110	1.450	1.450	6.000

Calculated H Value = 10.070 Critical H Value Table = 12.590
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

Shell deposition (mm), 96 hrs; mg ai/L
File: 4301sd Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 0 6 7 3 5 2 4 1
6	68	**1.400**	**1.400**	\
7	**110**	**1.450**	**1.450**	**. **
3	22	1.700	1.700	. . \
5	42	1.700	1.700	. . . \
2	15	1.750	1.750	. . . . \
4	31	1.950	1.950	. . . . . \
1	neg control	1.950	1.950	. . . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 4.100